AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Please insert the following replacement paragraphs at page 9:

Figure 11 is a schematic representation of another embodiment of a MHD stirrer in which the electrodes are transverse to the conduit's walls and they are labeled ~~A_i (i=.... 2, 1, 0, 1, 2,...)~~ 82a, 82b, 82c, 82d and 82e.

Figure 14 depicts schematically a continuous flow thermal cycler that can be used for PCR. ~~A1, A2, B1, B2, C1, C2, D1, and D2~~ 112, 114, 116, 118, 122, 124, 128 and 130 are electrodes. ~~The different shades of gray-scale denote zones maintained at different temperatures.~~

Please insert the following replacement paragraphs at page 11:

The basic building block of the controlled-flow MHD fluidic network is the conduit. Described generally with reference to Figs. 1a and 1b, the individual conduit **10** of the fluidic network has length L, width W, and height h. The conduit **10** may be capped, as depicted in Figs. 1a, 1b, 2a and 2b, or open from above as shown in Fig. 6. Moreover, conduits comprising a network of conduits may have the same or different shapes, lengths and sizes provided that the conduits are capable of bearing electrodes positioned suitably for the generation of Lorentz forces upon the application of a current or potential within a magnetic field. Suitable configurations include, for example, rectangular, as shown in Figs. 2a and 2b. Alternatively, the conduits may comprise straight, curved or slanted walls that in cross-section are square, trapezoidal, circular, oval, or any other such suitable shape or combination of shapes.

The network of conduits may be simple or complex comprising any combination of curved or straight conduits with few or many interconnections arrayed in either two or three dimensions. A network **60** comprising solely of straight conduits is shown in Fig.

7, and Fig. 5 depicts an example of a network <u>**30**</u> comprising a combination of straight and curved conduits. Further, Figs. 4, 5, and 7 provide schematic depictions of embodiments of relatively simple, two-dimensional networks. In Figs. 4 and 5, the individual conduits, similar in structure to the conduit depicted in Figs. 1a and 1b, are denoted with numbers 1-6 and 1-10, respectively. The network <u>**20**</u> can be connected to external supplies and drains denoted as a, c, and e in Fig. 4. Alternatively, fluid can circulate inside the network <u>**30**</u> without external links as shown in Fig. 5.

Please insert the following replacement paragraph at pages 12-13:

The conduits of the fluidic network are provided with at least one pair of electrodes (denoted in Figs. 1a and 1b as ~~C_U~~ <u>**11a**</u> and ~~C_D~~ <u>**11b**</u> of length L_e) positioned on opposing internal surfaces of the conduit. These electrodes, referred to as driving electrodes, define the region along the conduit in which an electric current or potential is applied. The driving electrodes may be positioned on the internal surfaces of the conduits in a variety of ways all of which are considered within the scope of the invention. Two such electrode configurations are depicted in Figs. 2a and 2b. Fig. 2a depicts an arrangement of electrodes comprising four individual electrodes <u>**12a, 12b, 12c, and 12d**</u> positioned along the corners of a conduit as shown in cross-section <u>in operational engagement with electrode controller **8**</u>. Fig 2b depicts an arrangement of electrodes comprising a pair of individual electrodes <u>**14a and 14b**</u> each covering the entire area of opposing sidewalls of a conduit <u>in operational engagement with electrode controller **8**</u>. The arrangement of electrodes as shown in Fig. 2b is a configuration that can provide a nearly uniform current density in a fluid within the conduit. Electrodes may also be used to control the shape of the velocity profile and, depending on the specific application, arrangements other than those shown in Figs. 2a and 2b may be preferable.

Please insert the following replacement paragraph at pages 13-14:

The driving electrodes themselves may be used to form virtual conduits, that is, conduits which lack physical walls for the containment of the fluid. Flow of fluid through a network comprising virtual conduits is spatially defined by the configuration of the electrodes on the substrate and controlled by the current or voltage applied across electrode pairs. In such networks, the electrodes may either protrude from, be flush with, and/or terminate beneath the surface of the substrate. Figs. 3a and 3b depict an example of a toroidal virtual conduit **16** and a straight virtual conduit **18** in which the "walls" of the conduits are the electrodes **17a, 17b, 19a, and 19b** themselves. Figs. 3a and 3b correspond, respectively, to a top view and a view in cross-section of the virtual conduits. Complicated patterns of electrodes may be readily manufactured using a variety of printing and lithographic techniques for applying the electrodes to the substrate.

Please insert the following replacement paragraph at page 18:

An exemplary MHD-controlled fluidic network **20** is shown in Fig. 4. The individual conduits comprising the network are denoted by the numbers 1 through 6 and the nodes are denoted with the letters a through e. Nodes a, c, and e communicate beyond the network or with reagent reservoirs and serve as sinks and sources. In an alternative embodiment, the network is not provided with any sinks or sources. Conduits which do not contain driving electrodes have hydro-magnetic conductivity set to zero. For the network depicted in Fig. 4, six equations relate the flow rate in a conduit to the pressure drop along that conduit's length and the potential difference across the electrodes. Additionally, mass continuity (Kirchhoff's law) requires that all the flow rates arriving at each node sum up to zero. When the potential differences across all the conduits and the pressures at the sources and sinks are given, these equations can be solved to obtain the flow rates in all the conduits.

Please insert the following replacement paragraph at pages 19-20:

In one embodiment as shown in Fig. 6, layer ~~A~~ **40** is the top layer that contains the flow conduits **42** and includes 1.1 mm wide x 1.7 mm deep flow conduits **42** and

soldering pads **44** using DuPont 6134 solderable conductors. The soldering pads **44** are connected through vertical vias **45** filled with DuPont 6141 via fill paste (not shown) to the various electrodes. While relatively large conduits are fabricated in this embodiment to facilitate easy flow visualization, similar networks may be fabricated having much smaller dimensions. Layer ~~B~~ **46** comprises the bottom wall of the conduits **48** and contains the electrodes **50** and some of the electrical leads **52** connecting to the electrodes. A more detailed layout of the electrodes shown in layer ~~B~~ **46** is provided in insert E. About 20 μm thick x 2 mm wide electrodes **50** made from DuPont 5734 gold paste are printed on the surface of layer ~~B~~ **46**. The gold electrodes **50** are aligned with the edges of the conduits **42** such that when layers ~~A~~ **40** and ~~B~~ **46** are attached, about 0.1mm of the widths of the electrodes **50** along each side of the conduits' vertical walls are exposed to the conduits **42**. Each conduit **42** is provided with a pair of driving electrodes **50**. A gap separates the driving electrodes in adjacent conduits. Silver conductors **56** made from DuPont 6145 conductor paste are printed on both layers ~~B~~ **46** and ~~C~~ **54** to facilitate the connection of each electrode to the soldering pads located on the surface of layer ~~A~~ **40**. All the leads are connected through vertical vias **45** to terminals located on the surface of layer ~~A~~ **40**. Layer ~~D~~ **58**, the bottom layer, contains additional leads (not shown). Subsequent to machining and printing, the individual parts are stacked, aligned, laminated, and co-fired to form a sintered, monolithic block. The device may capped with a cover plate or left uncapped to facilitate easy access to the channels and to enable dye injection for flow visualization.

Please insert the following replacement paragraph at pages 21-22:

Figure 7 depicts schematically a more complicated MHD network consisting of a plurality of wells ~~R~~ **62** and conduits **10** through which reagents, analytes, or chemicals may be pumped along any desired path and stirred, causing various interactions and/or reactions. Each of the conduits shown in Fig. 7 has a structure similar to the conduit depicted in Fig. 1. Analytes and reagents may be pumped from any of the wells, brought together, and mixed to interact and/or react with reagents pumped from other wells. The network may also facilitate combinatorial screening in which many processes are carried

out in parallel. Moreover, reaction and interaction products may be used in subsequent reactions or interactions in either pre-determined or feedback modes. The embodiment depicted in Fig. 7 can readily be expanded to a three-dimensional network allowing a much larger number of connections. These examples illustrate that MHD-controlled networks provide an easy, effective and inexpensive way of circulating fluids through microfluidic laboratory on a chip conduits.

Please insert the following replacement paragraph at pages 23-24:

In one embodiment, the MHD stirrer of the present invention comprises a conduit having at least one electrode disposed along the wall of the conduit, and at least two electrodes positioned within the conduit and away from the wall. In another embodiment, the MHD stirrer comprises at least two electrodes disposed along at least one wall, and at least one electrode positioned within the conduit and away from the wall. In a further embodiment, the stirrer has at least two electrodes aligned along at least one wall, and at least one electrode disposed along another wall. In yet another embodiment as shown in Figs. 1a and 1b, the MHD stirrer comprises a pair of electrodes **11a and 11b** disposed along the opposing walls **10a and 10b**, and at least two electrodes **$11c_i$** positioned within the conduit and away from the opposing walls. In this embodiment, the electrodes **$11c_i$** positioned within the conduit and away from the opposing walls **10a and 10b** may be aligned as shown in Fig. 1a along the centerline of the conduit's bottom. In still another embodiment, the MHD stirrer comprises a cylindrical chamber with an electrode disposed around its internal periphery and at least two electrodes positioned eccentrically inside the chamber. The placement of the one or more electrodes permits the generation of complex secondary flows including flows characterized by chaotic advection that is beneficial for mixing or stirring within a fluidic conduit or chamber. The conduits as described in all of these embodiments may comprise a conduit of the MHD-driven fluidic network or thermal cycler of the present invention.

Please insert the following replacement paragraph at pages 24-25:

In the embodiment shown in Figs. 1a and 1b, a conduit **10** of a controlled, MHD-driven microfluidic network is provided with a pair of electrodes **11a and 11b** disposed on opposing walls **10a and 10b** of the conduit and a series of electrodes denoted ~~A_i~~ **11c**$_i$ disposed along the centerline of the conduit and away from the opposing walls. Electrodes ~~A_i~~ **11c**$_i$ (where i = 1, 2, 3,....) are referred to as stirring electrodes. This particular implementation of the stirrer is described in greater detail in Qian, S., Zhu, J., and Bau, H. H., 2002, *A Stirrer for Magneto-Hydrodynamically Controlled Micro Fluidic Networks*, Physics of Fluids, 14 (10): 3584-3592 which is incorporated herein in its entirety.

Please insert the following replacement paragraphs at page 25:

In order to operate a MHD conduit as a stirrer, the electrodes intended for use in creating secondary flows are in operable engagement with at least one electrode controller such as, for example, a computer-controlled relay actuator. In one embodiment, relay-actuators combine both driving electrodes ~~C_U~~ **11a** and ~~C_D~~ **11b** into a single electrode C. When a potential difference is applied across the electrode pair ~~C-A_i~~ **C-11c**$_i$, circulatory motion of the fluid within the conduit is generated, with the fluid circulating around electrode ~~A_i~~ **11c**$_i$. When the electrode pair ~~C-A_1~~ **C-11c**$_1$ is activated for a time interval T_1, electrode pair ~~C-A_2~~ **C-11c**$_2$ for another time interval T_2, then electrode pair ~~C-A_1~~ **C-11c**$_1$ once again, and so on in a periodic fashion, chaotic advection is generated. As the magnitude of the period ($T=T_1+T_2$) increases, the chaotic region increases in size and complexity. In some circumstances, it may be advantageous to alternate the electrode potentials in a non-periodic fashion.

In demonstrating this effect, flow visualization experiments of the stretching and deformations of a dye blob were performed. Fig. 8 depicts computational and experimental results when a blob of dye **70** was inserted into the conduit **72** and the evolution of the dye was tracked over time. Both in experiment and theory, a rapid spread of the dye was observed indicating efficient stirring. By engaging a larger number of electrode pairs ~~C-A_i~~ **C-11c**$_i$, one can further extend the fraction of the conduit that participates in the mixing process. As electrodes may be readily patterned into various

shapes, electric fields may be induced in different directions. The interaction of such electric fields with the magnetic field can be used to induce secondary complex flows that may be beneficial for stirring and mixing.

Please insert the following replacement paragraph at page 26:

Stirring electrodes such as electrodes ~~A_i~~ **$\underline{11c_i}$** shown in the embodiment depicted in Figs. 1a and 1b may be located singly or in combination anywhere within conduit **10** provided they are away from either of the opposing walls **10a and 10b**. The electrodes ~~A_i~~ **$\underline{11c_i}$** need not to be aligned along the conduit's center. Although it is convenient to print the electrodes on the device's floor to avoid intrusion, one can also use other arrangements such as, for example, electrodes in the form of pins that protrude into the conduit.

Please insert the following replacement paragraph at pages 27-28:

Fig. 11 depicts a schematic representation of another embodiment of a MHD stirrer. As shown in Fig. 11, the stirring electrodes **82a, 82b, 82c, 82d, and 82e** are aligned perpendicular to the conduit's walls **80a and 80b**. By subjecting these electrodes to varying potential differences in the presence of a magnetic field, forces are generated that drive fluid flow in various directions in "virtual" conduits whose geometry is dictated by the positioning of the electrodes. An implementation of the stirrer is described in greater detail in Bau, H. H., Zhong, J., and Yi, M., 2001, *A Minute Magneto Hydro Dynamic (MHD) Mixer*, Sensors and Actuators B, 79/2-3, 205-213; and Xiang, Y. and Bau, H. H., 2003, *Complex Magneto Hydrodynamic, Low Reynolds Number Flows*, Physical Review Letters E, 68, 016312-1 – 016312-11, which are incorporated herein in their entirety.

Please insert the following replacement paragraph at page 28:

Fig. 12 depicts the deformation of an initially straight dye line resulting from the application of Lorentz forces by means of a MHD stirrer of the present invention. A thin

trace of dye **90** (Water Soluble Fluorescent Liquid Dye, Model 298-16-Red, Cole Palmer Instrument Company, Niles, Illinois, USA) is applied by means of a syringe across the cavity **92** and then a potential difference is applied across adjacent electrodes **94a, 92b, 92c, 92d, and 92e**. As a result of the application of the potential difference, fluid flow is induced in the cavity. The motion consists of rotating cells with the fluid moving up in one interval between two electrodes and down in the adjacent interval. Frame A in Fig. 12 depicts the line of dye initially inserted into the device. Depending on the polarity of the electrodes, the dye either moves upwards or downwards as shown in frame B of Fig. 12. After a few seconds, the polarity of the electrodes is reversed. Since diffusion is relatively slow and the flow is at a relatively low Reynolds number, the dye retracts its steps in almost a reverse fashion as shown in frame C of Fig. 12 and then starts deforming in the opposite direction as shown in frame D of Fig. 12. When the process is allowed to continue for some time, the dye traces the convective cells as shown in frame E of Fig. 12 in good qualitative agreement with theoretical predictions.

Please insert the following replacement paragraph at page 29:

Fig. 13 compares theoretical predictions shown in the left column and experimental results shown in the right column obtained in another implementation. Figure 13 depicts the deformation of an initially straight line of dye under various operating conditions. The top row, Figs. 13a and 13b, depicts the flow structure when only the odd-numbered electrodes are active. By alternating the potential difference across non-adjacent pairs of electrodes, it is possible to induce chaotic motion in the cavity **100**. For example, electrodes ~~A_{-2}, A_0, and A_2~~ **102a, 102c, and 102e** may be engaged for the time interval T_1, and then electrodes ~~A_{-1}~~ **102b** and ~~A_1~~ **102d** for the time interval T_2. By repeating this mode of operation, fairly complicated flows are generated and effective stirring is provided. The results of this mode of operation are depicted in the bottom row of Fig. 13, Figs. 13c and 13d.

Please insert the following replacement paragraph at pages 29-30:

Fig. 14 depicts one embodiment of the thermal cycler of the present invention. The cycler comprises a closed conduit loop **110** with electrodes aligned along opposing walls. Electrodes ~~A1~~ **112** and ~~B1~~ **114** are aligned along the inner wall of the loop **110**, and electrodes ~~A2~~ **116** and ~~B2~~ **118** are aligned along the outer wall of the loop **110**. An entry port **120** with electrodes ~~C1~~ **122** and ~~C2~~ **124** aligned along its opposing walls leads into the loop **110** and an exit port **126** with electrodes ~~D1~~ **128** and ~~D2~~ **130** aligned along its opposing walls leads out of the loop **110**. While the device shown in Fig. 14 has one inlet and a separate exit port, the cycler may be equipped with a single port or larger number of inlet and exit ports. In order to utilize the conduit loop depicted in Fig. 14 as a thermal cycler, different parts of the loop are maintained at different temperatures. The various temperature zones may be maintained, for example, with the use of electrical resistors or thermoelectric units (not shown). Fig. 14 depicts three thermal zones. It is contemplated as within the scope of the invention that a larger or a fewer number of thermal zones may be used as is suitable with reference to the particular application.

Please insert the following replacement paragraph at page 30:

At the beginning of operation, an electrical potential is applied to the electrodes such that the material is drawn into the loop. The polarities of either electrode pair ~~A1~~ **112** and ~~A2~~ **116** or electrode pair ~~B1~~ **114** and ~~B2~~ **118** are then reversed so that the material within the conduit loop is forced to circulate continuously around the loop. The particular choice of polarity will determine whether the motion is in the counterclockwise or clockwise direction. If necessary, the polarities and the magnitudes of the potentials applied to electrodes ~~C1, C2, D1, and D2~~ 110, 124, 128, and 130 may be adjusted so as to prevent the material within the conduit from leaving the loop. Also, if necessary, the direction of the flow in the thermal cycler may be periodically changed to minimize analyte migration in the electric field. As the material within the conduit cycles around the loop, it is exposed to different temperatures. In certain embodiments, this cycling between or among different temperature zones facilitates biological interactions such as, for example, those needed for PCR.

Please insert the following replacement paragraph at pages 30-31:

After the material within the conduit has completed the desired number of cycles around the loop, electrical potentials are supplied to the various electrodes so as to pump the reaction products out of the loop. The reaction products may be pumped either through the exit port **110** defined by electrodes ~~D1~~ **128** and ~~D2~~ **130**, back through the inlet port **120** defined by electrodes ~~C1~~ **122** and ~~C2~~ **124**, or split among any number of exit ports (not shown in the figure) so as to transport parts of the sample to different subsequent analysis paths. The embodiment of the MHD thermal cycler depicted in Fig. 14 may be readily integrated into a magneto-hydrodynamic network such as the one depicted in Fig. 6, integrated into a network in which fluids are propelled by other means than MHD, or used as a stand-alone device. In all three implementations, MHD stirrers of the present invention may be integrated into the MHD thermal cycler of the present invention to enhance efficiency.